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Original Article

Evaluation of nutrient content and in-vitro gas production of complete feed based on corn stover (*Zea mays*) supplemented by mimosa powder and myristic acid

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Abstrak

Tujuan: Penelitian ini bertujuan untuk mengevaluasi pengaruh suplementasi serbuk mimosa sebagai sumber tanin terkondensasi dan asam lemak tunggal yaitu *myristic acid* pada pakan lengkap berbasis jerami jagung dengan parameter produksi gas secara *in-vitro*. Penelitian ini telah dilaksanakan di Laboratorium Nutrisi dan Makanan Ternak, Fakultas Peternakan, Universitas Brawijaya, Malang dari bulan Agustus hingga Desember 2019.
Metode: Metode penelitian yang digunakan adalah Rancangan Acak Kelompok (RAK) dengan 4 perlakuan dan 3 ulangan/kelompok.
Hasil: Hasil penelitian menunjukkan perlakuan berpengaruh nyata (p<0,01) tehadap total produksi gas dengan P1 menunjukan nilai tertinggi yaitu 86,7 ml/500mg BK dan terendah pada P3 73,3 ml/500mg BK. Akumulasi produksi gas Metan dan karbon dioksida juga menunjukan perbedaan yang nyata (p<0,05) dengan nilai tertinggi pada P1 sebesar CH_4 (87657,73 ppm) dan CO_2 (436711,57 ppm) sedangkan P4 memiliki hasil yang terendah pada produksi CH4 sebesar (82863,07 ppm) dan P3 menunjukan produksi CO2 terendah yaitu sebesar (350287,72 ppm).
Kesimpulan: Kesimpulan yang didapat dalam penelitian ini yaitu penambahan serbuk mimosa sebagai sumber tannin terkondensasi dan asam lemak tunggal berupa Myristic acid pada pakan lengkap berbasis jerami jagung mampu meningkatkan nilai nutrisi ransum pakan lengkap dan dapat menurunkan produksi entrik gas Metan yang disebabkan proses degradasi pakan oleh mikroba rumen.

Kata Kunci: *Myristic acid*; Serbuk mimosa; Tanin terkondensasi; Gas metan

Abstract

Objective: The purpose of this research was to evaluate the effect supplementation of mimosa powder as a source of condensed tannins and a single fatty acid, myristic acid, in a complete feed based on corn stover (*Zea mays*) using the in-vitro gas production method. This research has been carried out at the Animal Nutrition and Food Laboratory, Faculty of Animal Husbandry, Brawijaya University. The time of the research was conducted in August until December 2019.

Methods: The experimental design used randomized complete block design by ANOVA consisting four treatments and three replications which were P1= a complete feed based on corn stover (*Zea mays*) as control Diet (CD) (40% corn stover + 60 % concentrate), P2= (CD) + Mimosa Powder(MP) 1.5 %/kg DM + myristic acid (MA)2% /kg DM, P3= CD + MP 1.5 % /kg DM + MA 3% /kg DM, and P4= CD + MP 1.5 %/kg DM + MA 4 %/kg DM.
Results: The results showed that the treatments affected total gas production ($p<0.01$). The highest value for total gas production was found in P1 (86.67 ml/500 mg DM) and the lowest was found in P3 (73.30 ml/500 mg DM). The results showed that gas production decreased concurrently with the increase of MA level. In vitro methane gas and carbon dioxide production was showed different ($p<0.05$) from the control treatment. The lowest concentration of methane production was in P4 (82863.07 ppm) and the highest concentration was in treatment P1 86530.89 ppm. The highest total carbon dioxide content was P1 (436711.57 ppm) and the lowest concentration was P3 (350287.72 ppm).
Conclusions: The results of the research concluded that the addition of mimosa powder and 3 different levels of myristic acid in a complete feed based on corn stover can increase the nutritional value of a complete feed and reduce the production of methane gas.

Keywords: Myristic acid; Condensed tannin; In-vitro gas production; Methane

INTRODUCTION

Corn plant (*Zea mays L.*) is a multifunctional crops commodity because it can be used as food, feed ingredients, bioethanol and a source of industrial raw materials. Data from the Indonesian Central Statistics Agency showed that corn production in the last 5 years increased by an average of 12.49 percent per year and harvested area per year which on average increased by 11.06 percent [1]. Corn stover is one of the main crop straws characterized by multi-source, wide distribution, high abundance, low cost, less competing usage, and great potential to be used as ruminant animal feed. Umiyasih [2] analyzed the chemical composition of corn stover and the results showed corn Stover is not a good roughage source and should not be used as the main roughage for ruminants because of their low contents of crude protein (CP) and high of fiber.

Ruminant nutritionists have studied technology feed processing named complete feed can be used on utilization of corn stover as feed to improve the productivity of ruminant livestock. The complete feed consisted of a mixture of 60% concentrate and 30% coarsely ground hay or straw (2.54 cm). Corn stover had high fiber content (74-86%) consequently low digestibility (40-53%) [3]. The study showed that feeding ruminants with higher fiber contain caused higher production of methane which is one of greenhouse gasses [4]. Ruminant methane emissions are of great importance since they allow for the quantification of two important aspects: greenhouse gas emissions and ingested energy losses by ruminants [5]. In recent years animal scientists have tested a number of chemical compounds to inhibit methane production in ruminants. Tannins are naturally occurring plant polyphenols and their main characteristic is that they bind and precipitate proteins [6].

Mimosa powder from wild plant Putri malu (*Mimosa pudica*) has reported contain high levels of tannins and it can be used as a condensed tannin (CT) source to protect essential amino acids for livestock from degradation by rumen microbes. Putri malu (*Mimosa pudica*) is a weed plant that grow out of along a river in Indonesia. Tannin at the level of 1% in the feed can give the best effect on fermentability of the feed, but it has not significantly suppressed methane production [7]. This makes tannin need another chemical compounds to inhibit methane production in the rumen.

Fatty acid have great potential as mitigation agents by supplemented into the feed [8,9]. Sitoresmi [10], has reported the addition of fatty acid by 7.5% can reduce the number of protozoa by 23.95% and reduce the amount of methane gas production by

18.51%. Ten types of fatty acids from CPO crude palm oil were observed such as laurate (C8), myristate (C14), palmitate (C16), palmitoleate (C16: 1), stearate (C18), oleate (C18: 1), linoleate (C18: 2), linolenic (C18: 2), arachidate (C20: 4), and gadoleate (C14: 0)[11]**.** Single fatty acid was reported to have a particularly high potential in suppressing ruminal methanogenesis [12]. Myristic acid is a by-product of making palm oil. The single fatty acid used in this research is fat in the form of triglycerides which is a by-product of the production of non-hydrogenated palm oil (CPO) and contains more than 99% high fat myristic acid with high melting point. Palm oil (*Elaeis guineensis Jacq*.) is one of the world's most produced and consumed oils. Palm oil and its derivatives are one of Indonesia's leading products with a contribution of 12.05% of the total value of Indonesia's exports [13]**.** Therefore, this research was conducted to evaluate effects of mimosa powder supplementation as a source of tannins and myristic acid as single fatty acid in in complete feed on corn stover base by the In Vitro gas method.

MATERIALS AND METHODS

Feed materials

Corn stover has a low CP that is equal to 5.13% so in this research it is treated as a complete feed. The processing of corn stover as a complete feed is intended as a feed that is sufficient nutrition for certain animals in a certain physiological level.

The constituent feed material used in the complete corn stover based feed in this study can be seen in Table 1**.** Corn stover and Putri malu (*Mimosa pudica*) ware obtained from research farm laboratory of Faculty of Animal Science, Brawijaya University. This area is located in East Java island Indonesia at an altitude of 506 m above sea level with an average annual rainfall and temperature of 369 mm and 22,7 – 25,1°C, respectively. Feedstuff for composition of the complete corn stover based feed were purchased from a feedstuff supplier in Malang city. The samples were oven dried at 50°C for 48 h then ground to pass through a 2.0 mm sieve. The dry samples were further ground to pass through a 1.0 mm sieve for the in vitro gas production experiments and chemical analysis. DM of feed component was determined by drying at 100 °C, organic matter by ashing at 550^{o}C for 4h and crude protein by the Kjeldahl technique and Van Soest fiber analysis.

Research design and preparation of complete rations

The experimental method used a group-randomized trials by ANOVA with 4 dietary treatments and 3 replications. The experimental diets were:

- P1: Corn Stover Based Complete Rations (Control Diet)
- P2: Control diet +Mimosa powder (MP) 1,5% /kgDM + Myristic acid (MA) 2% /kgDM
- P3: Control diet + MP 1,5% /kg DM + MA 3% /kg DM

Table 1. Proximate composition of Ingredient feeds used for in vitro cultivation experimental diet

No	Ingredient Diet content	DM	Ash*	OM*	CP*	CF*	EE*
1	Corn stover (*Zea mays*)	60.31	10.17	89.83	5.13	36.43	0.63
3	Coffee waste (*Coffea arabica*)	94.14	10.58	89.42	10.11	34.00	1.49
4	Rice bran (Oryza sativa L)	90.63	12.60	87.40	10.15	16.20	13.00
5	Tapioca by-Product (*Manihot esculenta*)	92.59	17.13	82.87	1.76	25.39	0.44
6	Soybean Meal (*Glycine max*)	93.53	8.38	91.62	47.53	4.04	2.57
7	Palm kernel meal (*Elaeis guineensis* Jacq.)	95.39	5.03	94.97	14.24	20.91	10.01
8	Copra Meal (*Cocos nucifera L.*)	95.69	7.77	92.23	22.12	21.78	2.45
9	Mimosa Powder (*Mimosa pudica*)	90.22	3.57	94.22	8.37	3.30	1.43
10	Urea	99.88	0.07	99.93	244.60	-	-
11	Molasse	78.47	15.44	84.56	4.54	-	-

*, DM% basis.

- P4: Control diet + MP 1,5% /kg DM + MA 4% /kg DM

In vitro gas production test

The In vitro gas-production technique more valid which compared with other laboratory techniques for predicting of animal performance and feed ruminant intake. Moreover, it is more efficient technique for determining the nutritive value of feeds containing anti-nutritive factors and for evaluating the microbial fermentation of ruminant feeds and its impact on fermentation products. The Rumen fluid was obtained from slaughterhouse. The samples were incubated in the rumen fluid in calibrated glass syringes following the procedures of Menke [14] as follows. 0.500 g dry weight of the sample was weighed in triplicate into calibrated glass syringes of 100 ml.

The syringes were pre-warmed at 39^{o}C before injecting 50 ml rumen fluid-buffer mixture into each syringe followed by incubation in a water bath at 39^{o}C. The syringes were gently shaken 30 min after the start of incubation and every hour for the first 10 h of incubation. Gas production was measured as the volume of gas in the calibrated syringes and was recorded before incubation (0) and 2, 4, 6, 8, 12, 24, 36 and 48 hours after incubation. Total gas values were corrected for blank incubation which contains only rumen fluid. At the end of incubation (48 h) gas collected for methane gas and carbon dioxide analyzed. Methane gas and carbon dioxide were measured with a Flame Ionization Detector (FID) in Agricultural Environment Research Institute.

Data analysis

The data from the experiment were compiled in the SPSS version 20 and Duncan's multiple-range test was subsequently conducted when there was a significant difference among dietary groups. Cumulative gas was expressed as ml of gas produced per 500mg of dry matter and corrected for blanks.

RESULTS

Chemical composition of feed

Evaluation of nutrient content of complete feed based on corn stover (*Zea mays*) supplemented by mimosa powder and myristic acid presented in Table 2.

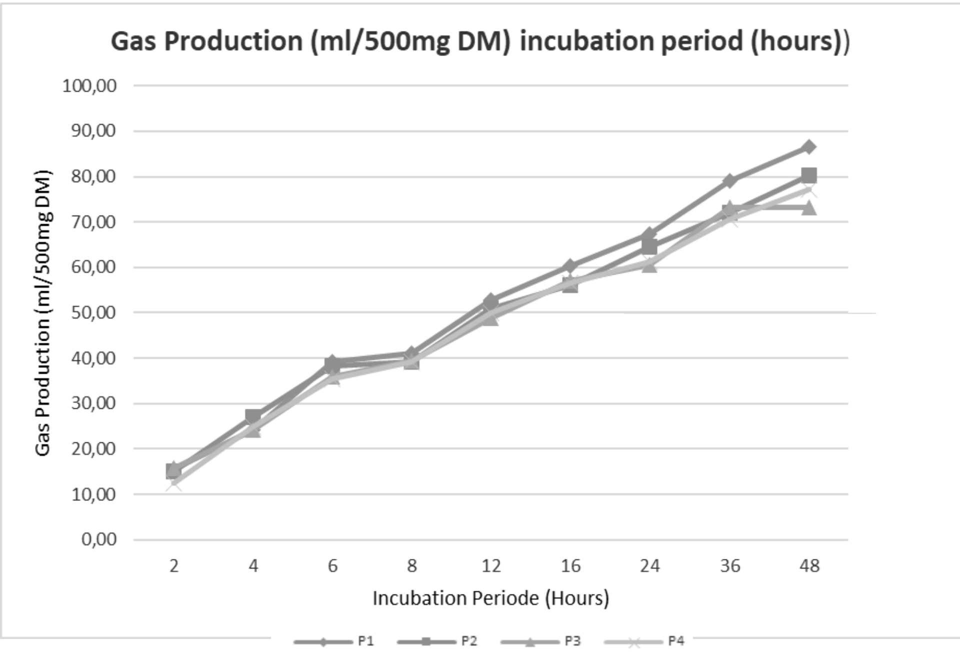

Figure 1. Graph of total gas production with an incubation time of 48 hours. P1, Corn stover based complete rations (Control Diet): P2, Control diet + Mimosa powder (MP) 1,5% /kgDM + Myristic acid (MA) 2% /kgDM; P3, Control diet + MP 1,5% /kg DM + MA 3% /kg DM; P4, Control diet + MP 1,5% /kg DM + MA 4% /kg DM

The crude protein content (CP) in the complete feed based on corn stover can be seen in the tables in P1, P2, P3, and P4 respectively 14.23%, 15.59%, 13.79%, and 14.09%. The content of crude fiber (CF) complete feed based on corn stover in this research showed a high number that ranges from 24% -24.53% while the ADF yield ranges from 31.43%-45.21% and NDF 46.45% -50.33%. The crude fat content of E in each treatment also showed a quite noticeable difference (Table 2) with the highest value at P4 7.12% and the lowest at P1 2.94%.

In vitro gas production

The value of total gas production, methane and carbon dioxide complete feed based on corn stover supplemented with mimosa powder and Myristic acid can be seen in Table 3.

Addition of Mimosa-Myristic acid powder with 3 different levels showed different gas production ($p<0.01$) at 16, 36 and 48 hours and different ($p<0.05$) at the 2nd hour, 6 compared with controls. The addition of tannins and 3 different levels of Myristic acid in complete feeds based on corn stover gave an effect ($p<0.05$) on the production of Methane. The pattern of increased gas production from complete feed based on corn stover with supplementation of condensed tannins and 3 different levels of myristic acid can be seen in Figure 1.

Figure 1 shows a graph of total gas production in all treatments increasing with increasing incubation time. Gas production will continue to increase as long as the substrate fermented by microbes is still available. Gas produced by rumen microbes during incubation is a product of microbial metabolism in degrading feed and also as a result of the buffering effect of artificial saliva (buffer solution) when VFA is produced.

Methane and carbon dioxide gas production

Methane and carbon dioxide gas production in this study are presented in ppm which is the result of methane gas analysis using the gas chromatography method. Data from observations of accumulation of methane gas production are presented in Table 3.

The results showed that the addition of myristic acid could significantly influence the reduction in methane gas. In Table 3 showed the In vitro methane gas and carbon dioxide production was showed different ($p<0.05$) from the control treatment. The lowest concentration of methane production was in P4 (82863.07 PPM) and the highest concentration was in treatment P1 86530.89 PPM. The highest total carbon dioxide content was P1 (436711.57 PPM) and the lowest concentration was P3 (350287.72 PPM).

DISCUSSION

Effect on chemical composition of complete feed based on corn stover supplemented by mimosa powder and myristic acid

The nutrient composition of corn stover relatively has low protein content CP 4.05 %, NDF 71.93 and ADF 41.36. Corn Stover substantially varied among different

Table 2. Proximate composition of experimental diet

Chemical composition (% DM)	Treatments			
	P1	P2	P3	P4
Dry Matter (DM)	93,44	93,82	93,53	93,78
Crude Protein (CP)	14,23	15,59	13,79	14,09
Crude Fiber (SK)	24	24,53	24,6	24,9
Extract Eter (EE)	2,94	4,07	5,44	7,12
Acid Detergent Fiber (ADF)	45,21	46,83	31,43	35,22
Neutral Detergent Fiber (NDF)	49,34	50,33	46,45	46,75

P1, corn stover based complete rations (control diet); P2, control diet + mimosa powder (MP) 1,5% /kgDM + myristic acid (MA) 2% /kgDM; P3, control diet + MP 1,5% /kg DM + MA 3% /kg DM: P4, control diet + MP 1,5% /kg DM + MA 4% /kg DM.

morphological fractions from the whole plant whereas stem rind had the lowest CP content (1.94%) and the highest ADF and ADL contents (47.59% and 8.32%,respectively) and leaf blade had the highest CP content (9.95%) and the lowest NDF and ADF contents (62.28% and 31.12%, respectively)[15]. Reasonable development and utilization of corn stover as feed source can be corrected by a combination of other feed stuff with high protein and mineral.

Nutrient supplementation both energy and protein together are intended to optimize microbial growth so that the utilization of feed can be optimized. Complete feed based on corn stover (*Zea mays*) is a reinforcing feed that is rich in protein and also energy to support the productivity of ruminants livestock. In the results of laboratory analysis of complete feed based on corn stover (*Zea mays*) containing crude fiber, ADF, and NDF which did not show differences among the treatments. The high content of carbohydrate fraction in complete feed based on corn stover (*Zea mays*) is influenced by the use of complete feed ingredients which constitutes agricultural by product. The feed ingredients contain cell wall components including NDF (*neutral detergent fiber*) and ADF (*acid detergent fiber*) which are high, causing the energy content in complete feed to be low**.** NDF is a constituent of fibrous cell walls consisting of cellulose, hemicellulase, lignin, silica and N cell walls. According Biyatmoko [16] the content of NDF and ADF in feed can affect the consumption of NDF anf ADF in livestock. ADF is a part of crude fiber consisting of lignin and silica, while NDF consists of cellulose, hemicellulose, and cell wall proteins. Fiber fraction, especially ADF (including NDF) can reduce the efficiency of energy use because it contains limited energy to maintain microbial growth; reduce the availability of nutrients in foods that contain high fiber; and tends to speed up rumen filling and limit feed consumption [17]**.**

The Crude fat (EE) content correlates with the addition of Myristic acid levels in the treatment. The use of Myristic acid in this study used a different level with the highest treatment at the 5% level. The level of fat in the feed if it is too high will cause a negative effect of fat on the degradation of feed by microbes in the rumen [18]**.** Meat from ruminant animals contains higher saturated fatty acids compared to non-ruminant [19]**.** One effective strategy for increasing the productivity of beef cattle and at the same time increasing the composition of unsaturated fatty acids in meat products is through supplementation of unsaturated fatty acid sources from plants [20]**.** The addition of oil to the ration has several benefits, such as increasing ration energy [21] and increasing the efficiency of energy use by inhibiting methanogenesis as a defaunation agent [22].

Effect on in vitro gas production of complete feed based on corn stover supplemented by mimosa powder and myristic acid

Gas production (ml/500 mg), which reflects the apparent substrate degradability**.**

Table 3. The value of gas production in incubation 2, 4, 6, 8, 12,16, 24, 36, 48 hours and gas production of methane (CH_4) dan (CO_2)

Treatments	Gas Production (ml/500mg DM) Incubation Period (hours)									CH_4 (mg/l)	CO_2 (mg/l)
	2	4	6	8	12	16	24	36	48		
P1	15,49^b	24,2	39,1^b	41,0	52,8	60,4^b	65,4	79,1^b	86,7^c	87657,73^b	436711.57^c
P2	15,14^c	27,0	38,2^b	39,3	51,0	56,2^a	61,5	72,0^a	80,2^{ab}	84640,33^a	369471.17^b
P3	15,63^c	24,1	35,8^a	39,3	48,9	57,1^a	60,6	73,2^a	73,3^a	82863,07^a	350287.72^a
P4	12,55^a	24,9	35,4^a	39,3	49,9	56,6^a	61,2	70,7^a	77,1^b	80617,43^a	373799.59^b

^{a,b},different superscript letters in the same column show significantly different effects ($p < 0.01$) on the 16 and 36 hour production and the 2, 6 gas production while for In vitro methane and carbon dioxide production shows an influential treatment significant ($p < 0.05$).

The addition of tannins causes the protein in the feed to be protected from degradation so that it will directly inhibit the production of gas which is a by-product of the fermentation process of feed nutrients. This is in accordance with research conducted by [23] states that the presence of tannins can reduce gas production in the In Vitro fermentation system due to the interaction of tannins with feed components that contribute to gas production, especially protein and fiber. Fluctuations in gas production occur at 24 and 48 hours of incubation, due to the reduced amount of ingested feed material. Inhibition of bacteria that occurs due to the use of tannins also influence this[24].

Production gas and the model parameters are shown in Figure 1, we found that Gas Production was decreased ($P<0.01$) in response to Mimosa-Myristric powder supplementation during the whole in vitro incubation period. The graph demonstrating that Mimosa-Myristric notably contribute to the altered rumen fermentation. A lower gas productions indicate a lower nutrient degradation in the rumen, which confirmed the results of our in vitro degradability trial [25]. Thus, we can conclude that the supplementation of complete feed based on corn stover with Mimosa-Myristric could be beneficial for the animal performance, as they increase the amount of digestible 'bypass'nutrients. These findings and assumptions should be confirmed with additional in vitro and in vivo studies.

Effect on methane and carbon dioxide gas production of complete feed based on corn stover supplemented by mimosa powder and myristic acid

The results showed that the addition of myristic acid could significantly influence the reduction in methane gas. The results of the study are in accordance with Odongo [25], the addition of Myristic acid can reduce the production of methane gas without having an influence on feed consumption and milk production.

Mimosa powder in this study was used as a tannin source using only one level as a positive control but the use of mimosa powder also functions as a reinforcing agent for defaunator [26]. Tannins inhibit protease enzymes and enzymes in the transport of the bacterial cell sheath and inactivate the function of genetic material in bacteria. Tannins can constrict bacterial cell walls so they can interfere with cell permeability and stunted growth [27]. The content of myristic acid in vegetable oils has a significant effect on the reduction of methane gas through the mechanism of the body's protozoan coating so that the protozoan cannot pass metabolic activity which causes the protozoan to die in the rumen. According to Jordan [28] a decrease in protozoan population due to the defaunation process caused a decrease in symbosis between protozoans and methanogens, thereby reducing the availability of hydrogen for methane gas formation in the rumen. The secondary compounds of tannins and the single fatty acids myristric acid found in the feed affect the growth of rumen microorganisms, especially fiber degradation microorganisms. The reason for suppressing methane production of fats could be through a direct influence on the rumen methanogenic microbes. Provision of vegetable oil has the greatest effect on the average number of protozoa. The addition of medium chain fatly acid (MCFA) in the form of a single fatty acid causes a decrease in ciliated protozoa to reach 99.8 [29].

CONCLUSION

The conclusion obtained in this research is the addition of mimosa powder as a source of condensed tannin with low levels and 3 different levels of single fatty acids in the form of Myristic acid in complete feed based on corn stover (*Zea mays*) can increase the nutritional value of complete feed rations and can reduce the production of methane gas enteric due to the process degradation of feed by rumen microbes.

CONFLICT OF INTEREST

The authors declare no conflict of interest with any financial organization regarding the material discussed in the manuscript. The funders had no role in the design of the study;

in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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